Endoscopic Testicular Biopsy in Birds: Technique and Histopathological Results

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Introduction

Infertility is a common problem in avicultural collections, and can be defined as the inability to conceive or to induce conception. Generally speaking the causes of psittacine infertility are differentiated into 2 groups: medical and non-medical.

- **Medical** causes of avian infertility include lameness or orthopedic problems, ocular problems, malnutrition, disease of the reproductive tract, systemic diseases, cloacal abnormalities, hypothyroidism and toxicoses.

- **Non-medical** causes include immaturity, sexual inexperience, inappropriate perches, disturbances in or around the aviary, inappropriate nesting site and material, lack of flock stimulation, inbreeding/genetic, mate incompatibility, homosexual pairings, and heavy cloacal feathering.

If an "infertile" male bird, or a male whose mate is constantly, or mainly, producing infertile eggs is not showing any signs of disease, or when nothing relevant can be pointed out after a complete diagnostic process, and/or when the semen eventually collected does not contain any spermatozoa, or contains a very low number of vital sperms, a testicular biopsy can be indicated to reach a causative diagnosis. This can possibly help figuring out a treatment, and, more often giving a prognosis of the case.

Endosurgical Technique

Endoscopic testicular biopsies must be performed under general anesthesia. Anesthetic of choice is isoflurane. In the authors experience, patients do not need to be intubated because of the short surgery time (between 5 and 10 minutes). The patient is placed in right lateral recumbency, with the wings extended dorsally. The left leg is flexed cranially, while the right leg is maintained in its normal position. Both left and right lateral approaches were used to inspect both sides of the

coelemic cavity and airsac system. An entry site caudal to the femur, as described by Taylor, is preferred to a cranial approach, when performing a left lateral celiotomy, while a prefemoral approach seems smoother, when approaching the right side.

Bilateral testicle biopsies can be performed either with bilateral surgical access, or with a single left-side approach.

Before taking the biopsy, a thorough endoscopic evaluation of the main organs and especially of the gonads is a must. This can is performed with a 2.7-mm 30˚ offset rigid endoscope inserted into an examination sheath with an instrument channel.(Karl Storz GmbH, Tuttlingen, Germany). Illumination is provided by a 250W cold light source and a fiberoptic cable.

A small incision is made in the skin where the caudal thigh muscle crosses the last rib.

The abdominal muscles are bluntly dissected, either with an Adson, or with a small, curved mosquito forceps. The endoscope is inserted with its operating sheath into the abdomen, and the testicle can be visualized.

The flexible 5-Fr elliptic biopsy forceps is then inserted into the working channel for the biopsy of the testicle.

It is important to remember that the testes are extra-peritoneal organs, and they are covered and suspended by the mesorchium, that must be incised with the biopsy forceps, or with the scissors to reach the testicle. Actually an intact peritoneum could potentially lead to excessive pulling during biopsy, resulting in the tearing of local blood vessels. To avoid this complication, "fix" the bite with the biopsy forceps and use the examination sheath as a tool to keep the testicle in position. This can be achieved by pulling the biopsy forceps while gently pushing the examination sheath against the testis.

The testicle should be approached to allow the open jaws of the biopsy forceps to be perpendicular to the testicle's longer axes. The greater curvature of the testicle in this orthogonal approach makes it easier to snip a biopsy sample with minimal pulling. In addition, the forceps should be perpendicular to the surface of the testicle. A right testicular biopsy can also be performed from a left lateral approach. In this case, the air sac, the dorsal mesentery and the visceral sheet of peritoneum must be cut with endoscopic scissors to reach the right testicle. The biopsy forceps is passed through the openings created in this way, and the right testicle is accessible for biopsy.

Results

Birds that underwent testicular biopsy, were belonging to two different groups, and were biopsied in two different steps:

- healthy adult birds that authors used for developing the surgical technique and sharpening the pathologist's skills in reading and interpreting the biopsies;
- birds that had not being reproducing for at least two, or three consecutive years, when no any other possible reasons for this poor breeding performance could be pointed out, even after a thorough diagnostic work-up. This took into consideration the individual patient, its mate, and the breeding history of the pair. From each patient, after a complete physical examination, a thorough blood testing was run, including CBC, AST, UA, TP, LDH, antigen and antibody psittacosis. Further cloacal bacteriology and PCR for Circovirus, and serology for Polyomavirus were done.

A - Surgical Results:

So far, we examined 70 testicular biopsies from 61 birds, belonging to 31 different *taxa*. The systematic distribution of the examined birds was: 25 Amazons, 21 Macaws, 6 Conures, 6 Cockatoos, 2 African parrots, and 1 Brotogeris. No accidents were noted during, or immediately after testicular biopsy, but repeated endoscopic examination of some birds between 20 and 60 days post-surgery showed that biopsy can induce permanent anatomical damage to the testicle in some birds. In fact, two birds were observed with post-operative scars at the biopsy site, of the testes. The biopsy site in the left testicle was still evident in both birds at re-evaluation. Our observations suggest that small birds, or those birds with relatively small testicles. may be at greater risk, and this risk appears to be greater if the size of the biopsy is large relative to the testicle.

On the other hand, while testicular scars were observed in these two birds, no histologic changes could be pointed out in the testicles.

The other birds that were re-evaluated after having been biopsied, healed perfectly, suggesting that avian testes heal easily after biopsy, in the great majority of the cases.

B - Histological results

The lack a reasonable source of information about the histological interpretation of avian bioptic samples made the first attempts somehow challenging. Also it is important to remember that testicular biopsies are always made out of the breeding season. This in order to avoid the leaking of the testis content into the coeloma and also disturbances to a bird pair during the breeding season, before a diagnosis of infertility is made. As experience grew, a variety of different lesions were observed, and the following diagnosis were made:

- normal "atrophy" at the end of the breeding season, " testicular atrophy,
- chronic orchitis,
- granulomatous lesions,
- testicular degeneration.

Discussion

Infertility is a frequent problem in aviculture. Semen analysis can have some value, but generally does not provide an etiologic diagnosis, especially when no sperms are present in the ejaculate. Further it will not determine whether infertility is correctable or not, while testicular biopsy may provide a diagnosis.

Also, it must be noted that semen collection is performed during the breeding season, while biopsy should be performed at the end of it, because during the culmination phase, when testicles reach their maximum size, testicular biopsy can lead to partial leakage of tissue into the coelem. This means that most testicular biopsies show either the normal atrophy at the end of the season, a physiologic condition in wild birds, or pathologic conditions.

Proper technique is important during testicular biopsies to avoid unnecessary damage and to ensure a good sample. When the endoscope is placed too close to the testis, it does not allow proper handling of the forceps and may prevent a good visualization of the important structures surrounding the testicle.

Unless focal lesions can be visualized endoscopically, it is always better to take the biopsy be taken from the middle, cranial portion of the testis along its ventral surface, because this location will limit the risk of damage to the epididymis and/or the ductus *deferens.*

Even if some gross lesions of the testis, such as irregular surface, altered subcapsular blood supply, and different sized testicles were observed endoscopically, it is not yet possible to correlate them with the diagnosed histologic changes.

Testicular biopsy can be a useful tool to deepen the understanding of male birds fertility problems, but several facts must be understood and studied, if this technique has to be used in its diagnostic completeness.

Firstly, the avian testis differs from the mammalian one, both anatomically and histologically.
Size, color and shape of the testes may vary with age and also between species. Dark coloration and bicolored testes are not considered.

Also active and fertile testicles may show a wide variation in shape and size and therefore a simple endoscopic observation of the avian testicle, is not enough to judge its physiologic status, especially if the bird is showing poor breeding performance, or infertility. Further, the hormonal regulation of the sexual cycle in birds is not fully understood, which may be of significance with respect to species-specific differences, as well as by the pathologist perspective. In fact seasonal variations can be so striking that some experience is needed to interpret the development stage observed in the testicular tissue.

For example, the immature or inactive testicle is black because of a large number of melanocytes. When these birds become sexually active their testicles may turn grey and possibly even white from the increased volume of the seminiferous tubules.

Starting from these points of view, authors developed a complete protocol to screen male birds to be selected for testicular biopsy: in this way they hoped to complete the biopsy with the most exhaustive clinical history, trying to help the pathologist discriminate between what was really pathologic and what could be depending on other facts (i.e. species, age, season, presence of sexual stimuli, etc.).

The experience the authors gained with this study suggests that a merely visual evaluation of a testicle that looks endoscopically normal is not possible, if birds are showing low breeding performance. Semen collection and analysis seems a very promising tool, but although this technique is quite successful in some avian species (i.e. poultry, cranes, birds of prey and small psittacines), its success is still unpredictable in larger psittacine species. Further these two techniques are not to be considered competitive, but they are complementary, as semen collection can, when successful, give interesting results during the breeding season, but testicular biopsy is the next step, in the case of a negative answer from semen analysis.

Suggested reading

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